WHEELER. Alternaria in Nyasaland.

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INVESTIGATIONS ON ALTERNARIA LEAF-SPOT OF FLUE-CURED TOBACCO IN NYASALAND

by

B. E. J. WHEELER

Commonwealth Mycological Institute
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By B. E. J. Wheeler,
Colonial Pool of Plant Pathologists,
Commonwealth Mycological Institute

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INTRODUCTION

Alternaria leaf-spot (or brown spot), caused by A. longipes (Ell. & Ev.) Mason, is recognised as one of the most serious diseases of flue-cured tobacco in the Central African Federation. It was first reported in Southern Rhodesia by Hopkins in 1931 and subsequently there were severe epiphytotics in several districts within the territory (Hopkins, 1939, 1956). In more recent years, the disease has resulted in considerable losses in the Kasungu district of Nyasaland where estates for growing flue-cured tobacco were opened in 1950-1. The present investigations were carried out, at the request of the Nyasaland Government, in this district during 1954-5 and 1955-6 tobacco seasons.

II. SURVEY OF ALTERNARIA LEAF-SPOT IN THE KASUNGU DISTRICT

During the 1954-5 and 1955-6 seasons a survey of lands planted with tobacco in the Kasungu district was made to assess the incidence of Alternaria leaf-spot. At the same time information was collected from farmers and from the Department of Agriculture relating to the two previous seasons. In order to compare disease incidence over the four seasons for which data were available only two categories of disease incidence were used — moderate or severe infection and slight and no infection.

In describing the type of land planted with tobacco the following terms are used in the sense indicated.

First year land	Land not previously used for any crop, planted with tobacco in the season under review.
Second year land	Land on which tobacco was grown during the season preceding that under review and also in the season under review.
Reverted land	Land on which a crop other than tobacco was grown or on which no crop was grown in the season preceding that under review, although it may have formerly been used for tobacco.

(a) Disease incidence

The incidence of Alternaria leaf-spot in the Kasungu district over the four seasons 1952 to 1956 is summarised in Table I. The figures show that there was a

TABLE I

The incidence of Alternaria leaf-spot in the Kasungu district of Nyasaland during the 1952-3, 1953-4, 1954-5, and 1955-6 tobacco seasons

Season	1952-3	1953-4	1954-5	1955-6
Total acreage planted	463*	643	646	764
Total acreage severely infected with Alternaria leaf-spot	285	250	120	107
percentage of crop infected	61·2	38·9	18·6	14·0

*not a complete record for the district

marked reduction in the incidence of Alternaria leaf-spot during the latter two seasons compared with the previous two seasons. Thus a total of 38·9 per cent. of

the crop was either severely or moderately infected during the 1953-4 season and probably a higher percentage on the overall crop during the 1952-3 season, whereas during the 1954-5 and 1955-6 seasons it was 18·6 and 14 per cent., respectively.

Although only a crude assessment of disease incidence was possible, a detailed examination of the survey data indicated a number of factors which were associated with severe outbreaks of Alternaria leaf-spot. These are examined in the following section.

(b) Factors affecting disease incidence

Type of land. The severity of Alternaria leaf-spot has been most marked where second-year land has been used. An analysis of disease incidence in the Kasungu district in relation to the use of second year land (Table II) shows that 90·2 per cent. of all tobacco planted on such lands during the years 1952 to 1956 was seriously affected by the disease. For first year and reverted lands combined the corresponding figure is only 19 per cent.

TABLE II

The use of second year lands in relation to the incidence of Alternaria leaf-spot

Season	Number of second year lands planted	Number of second year lands severely infected	Number of other lands planted	Number of other lands severely infected
1952-3	18	18	27	9
1953-4	11	11	39	11
1954-5	11	6	54	6
1955-6	1	1	69	10
Totals	41	37	189	36
Percentage of plantings infected		90·2		19·0

Probably two factors operate here,

(1) The fungus can overwinter on tobacco debris (stalks, leaves, capsules, etc.). Any such material which is not destroyed provides a source of inoculum for possible infection in the following season and is potentially more dangerous if tobacco is again planted in the same field.

(2) Correct fertilization of second year land is more difficult particularly with regard to the nitrogen status of the soil. Misjudgement may result in a marked unbalance between the nitrogen, phosphate, and potash available, which in turn produces plants of "rank" growth which are more likely to be severely infected with Alternaria leaf-spot.

Type of soil. There were indications that the type of soil affected the subsequent incidence of disease. During the 1952-3 and 1953-4 seasons, some tobacco was planted in fields where the soil was of a sandy clay loam type. All tobacco grown on this soil was severely attacked by Alternaria leaf-spot. The reason for this is not known but there were indications that the fertilizers used were unsuitable and that an unbalanced relationship between the nitrogen, phosphate, and potash available to the plant resulted. This, in turn, produced tobacco deficient in one or more nutrients and highly susceptible to infection by A. longipes.

Fertilization. Fertilization of tobacco lands has an effect on disease incidence in so far as it affects nutritional status of the tobacco plant.

During the four years, 1952 to 1956, fertilization practices in the Kasungu district were considerably modified. The more important changes were as follows:

(1) Fertilizers formerly used, which contained high amounts of nitrogen in relation to their phosphate and potash content (e. g. "A" mixture, 6:10:8*) were replaced by those which contain more phosphate and potash so that the amount of nitrogen relative to these two nutrients is lower (e. g. "K" mixture, 2:18:15; "H" mixture, 3:18:15; "V" mixture, 4:18:15).

(2) There was a tendency to decrease the amount of nitrogen added during fertilization. In the 1952-3 season 41 lands received 25 lbs. or more of nitrogen per acre, whereas in the 1954-5 and 1955-6 seasons only 11 and 10 fields, respectively, received this amount of nitrogen.

(3) There was also a decrease over the last three seasons in the number of plantings which received additional applications of fertilizer as side-dressings. In the 1953-4 season 28 plantings were side-dressed, 22 in 1954-5 and 13 during the 1955-6 season.

(4) The placement of fertilizer as a "dollop" near the plant was replaced by application in a double band along the ridge or in a ring around the plant at the crown level.

Analyses of the survey data with regard to the first three changes mentioned above have been made and are summarised in Tables III, IV, and V.

TABLE III

The use of high applications of phosphate (> 100 lb. P_2O_5/acre) and potash (> 90 lb. K_2O/acre) in relation to the incidence of Alternaria leaf-spot

Season	Plantings given			
	High phosphate and potash		Low phosphate and potash	
	No. planted	No. severely infected	No. planted	No. severely infected
1952-3	Nil	Nil	45	26
1953-4	28	9	17	8
1954-5	38	4	26	8
1955-6	68	8	8	3
Totals	128	21	96	45
Percentage of plantings severely infected	16·4		46·9	

These indicate that each change had an effect in reducing the severity of Alternaria leaf-spot in the district. Thus during the four seasons under review, only 16·4 per cent. of those plantings which received high applications of phosphate and potash (100 lbs. P_2O_5 and 90 lbs. K_2O or more per acre) became seriously infected with

* 6:10:8 indicates the proportions of nitrogen, phosphate, and potash in the mixture. Each 100 lbs. of this fertilizer mixture contains, 6 lbs. of nitrogen, 10 lbs. phosphate as P_2O_5, and 8 lbs. potash, as K_2O.

Alternaria leaf-spot, whereas 46·9 per cent. of the plantings which received lower applications were seriously attacked. Similarly, during the same period, 55·2 per cent. of all plantings receiving 25 lbs. or more nitrogen per acre were seriously infected with Alternaria leaf-spot whereas plantings receiving lower amounts of nitrogen were correspondingly less affected by the disease.

TABLE IV

The rate of nitrogen application in relation to the incidence of Alternaria leaf-spot. (Summary of the 1952-3, 1953-4, 1954-5 and 1955-6 seasons.)

Rate of N. in lbs./ac.	0-12	13-18	19-24	25-30	over 30
No. plantings to which this was applied	25	43	69	58	20
No. plantings severely infected	2	6	15	32	10
Percentage of plantings infected	8·0	14·0	21·7	55·2	50·0

TABLE V

The use of additional applications of fertilizer in relation to the incidence of Alternaria leaf-spot

Season	Number of plantings			
	Side-dressed	severely infected	Not Side-dressed	severely infected
1953-4	28	12	17	5
1954-5	22	7	42	7
1955-6	13	7	58	4
Totals	63	26	117	16
Percentage of plantings infected	41·3		13·7	

With regard to additional fertilizer, during the last three seasons surveyed, 41·3 per cent. of the plantings which received side-dressings were seriously infected with Alternaria leaf-spot as compared with 13·7 per cent. of those plantings which received no side-dressings. It is probable that the increased likelihood of the crop "greening-up*" after the application of a side-dressing is the most important factor here. Observations have indicated that if a small amount of Alternaria leaf-spot is present and the crop "greens up", then the danger of an epiphytotic developing is considerable. No data are available concerning fertilizer placement in relation to disease incidence but certain experiments carried out at the Tobacco Research Board of Rhodesia and Nyasaland (Stinson, 1953) suggest that applications in a double band along the ridge or in a ring round the base of the plant, are to be pre-

*"Greening-up" is the term commonly given to the darkening of the leaves associated with a sudden increase in the availability of nitrogenous nutrients.

ferred to application in "dollops" near the plant.

Time of planting. The exact effect of the time of planting on the incidence of Alternaria leaf-spot is difficult to assess since if an early planting becomes severely infected adjacent later-planted tobacco is subjected to an abnormally high spore-load and this may profoundly affect the degree of infection in that planting. An analysis of the survey data (Table VI) shows a slight increase in disease incidence in plantings made after the 14th December but in any individual season (e.g. 1954-5) this may not necessarily be true.

TABLE VI

The relationship between time of planting and incidence of Alternaria leaf-spot in the Kasungu district.
(Summary of the 1953-4, 1954-5, and 1955-6 tobacco seasons.)

Time of planting	Nov. 15-30	Dec. 1-14	Dec. 15-31	Jan. 1-14	Jan. 15-31
No. plantings made	26	76	28	27	5
No. plantings severely or moderately infected with Alternaria leaf-spot	5	15	7	6	1
Percentage of plantings infected	19·2	19·7	25·0	22·2	20·0

Much appears to depend on the incidence of rainfall particularly during the ripening of the tobacco. In general, where ripening has coincided with frequent showers of rain there has been a tendency for the tobacco to be more severely infected (Table VII).

TABLE VII

The incidence of Alternaria leaf-spot in relation to rainfall and ripening of the tobacco.
(Data collected from one estate in the Kasungu district.)

Field	Planting date	Number of days during ripening* when rainfall exceeded 0·1 in.	Incidence of Alternaria leaf-spot
1	1.12.53	24	severe
2	5.12.53	19	severe
3	5.12.53	18	severe
4	10.12.53	18	severe
5	12.12.53	18	moderate-severe
6	10.12.53	13	slight
7	19.12.53	11	slight
8	31.12.53	7	slight

*from the first to the last reapings

During such a period of rain not only do conditions appear to be highly favourable for the spread of the fungus causing the disease but also the tobacco reaches its

final maturity more slowly, thus remaining in a highly susceptible condition for a longer period.

Discussion. Clearly a number of cultural practices have influenced the severity of Alternaria leaf-spot through their effect on the nutrition of the tobacco plant. In the Kasungu district the following have been associated with severe outbreaks of the disease:

(1) use of second year land,
(2) use of heavy (sandy clay loam) soils,
(3) applications of nitrogen in excess of 24 lbs. per acre,
(4) application of less than 100 lbs. phosphate (P_2O_5) and/or less than 90 lbs. potash (K_2O) per acre,
(5) use of additional application of fertilizer as side-dressings.

This does not necessarily imply that employing any one or a combination of these treatments will inevitably lead to a severe outbreak of Alternaria leaf-spot. The indications are, however, that by doing so the likelihood of incurring a serious infection is increased. Of 73 plantings severely affected by Alternaria leaf-spot which were recorded in the Kasungu district over the years 1952 to 1956, 67 were subjected to one or more of the five adverse treatments mentioned above. Six only were not. Of these six plantings, four were immediately adjacent to plantings severely infected with Alternaria leaf-spot and two adjacent to a land where considerable quantities of tobacco trash were lying on which A. longipes was sporulating freely, so that an abnormally high spore load is one possible explanation of the severe infections. The survey also showed, however, that there were plantings which did not become severely infected in spite of adverse cultural treatments. Here, lack of inoculum with or without unfavourable weather conditions for infection are the most likely causes. Further consideration of these factors is given in later sections.

III. FIELD AND LABORATORY EXPERIMENTS ON THE DISEASE AND THE CAUSAL ORGANISM

(a) Sources of Inoculum

Overwintering of A. longipes on tobacco debris. Harvesting of the flue-cured tobacco crop in Nyasaland normally ends by the beginning of April and planting of the next crop starts at the end of the following November. This coincides with the rainy season. In the intervening period there is little or no rain and conditions become increasingly unfavourable for vegetative growth. Previous experiments by Riley (1950) have indicated that A. longipes can survive during this period on tobacco debris (stalks, leaves, capsules, etc.) which is not adequately destroyed. The importance of this was investigated by collecting in mid-October, 1955, samples of tobacco debris from a number of lands in the Kasungu district on which tobacco had been grown in the 1954-55 season. These samples were examined for the presence of A. longipes and the viability of this determined.

The following are the methods used:

(1) Portions of dry trash were mounted in lactophenol, cleared by heating and examined under the microscope for the presence of spores.

(2) Portions of trash were placed in Petri dishes containing damp filter paper and allowed to remain at room temperature for two days. Small pieces were then examined as in (1).

(3) A quantity of each sample of dry trash was crushed and thoroughly mixed with a small quantity of distilled water. The resulting suspension of material was filtered through folded muslin and the filtrate subsequently centrifuged at 5000 r.p.m. for five minutes. The sediment was resuspended in about 1 ml. of distilled water. The following treatments were then carried out:

(i) Drops of the suspension were placed in cavity slides and incubated in Petri

dishes at 28°C. for 24 hours. At the end of this period the drops were examined for germinating spores of A. longipes.

(ii) Drops of the suspension were placed in sterile Petri dishes and maize-meal agar containing 2 per cent. potassium thiocyanate to suppress bacterial growth, added to these. The plates were incubated at 28°C. for three days and then examined for colonies of A. longipes. The plates were re-examined at intervals.

(iii) Drops of the suspension were placed on leaves of 15-week old seedlings (var. Delcrest) taken from the seedbeds. These were incubated in Petri dishes containing damp filter paper at 28°C. for three to four days and then examined for infection by A. longipes.

(4) In addition to the above, pieces of moistened trash were placed on seedling leaves as in (3, iii) and these subsequently examined for infection.

The results of these tests are summarised in Table VIII.

TABLE VIII

Survival of A. longipes on tobacco trash from the Kasungu area of Nyasaland

Estate and Field No.		Microscopic examination		Spore Germination	Growth on agar	Tobacco inoculations	
		Dry trash	Moist trash			Centr'd material	Moist trash
Chingaliwa	1	+	+	+	+	+	-
"	2	+	+	+	+	+	-
Chilanga	17	+	+	+	-	-	+
"	42	+	+	+	+	-	+
"	43	-	+	+	+	+	+
"	48	+	+	-	+	+	-
Cholo-Kasungu	1	+	+	+	+	+	+
"	2	+	+	+	+	+	+
Kamphuru	1	-	-	-	+	+	+
"	6	+	+	+	+	+	+
Lisasadzi	9	+	+	+	+	+	+
"	15	+	+	+	+	+	+
Manyani	2	+	+	+	+	-	-
Nchenga 1	1	+	+	+	+	+	+
Sable-Creek	1	+	+	+	+	+	+
"	2	+	+	+	+	+	+
"	3	+	+	+	+	+	+

Although there was a certain amount of variability in the tests, there is little doubt that A. longipes had survived on the tobacco trash and was in a viable condition. All samples were collected from the soil surface and almost certainly this material was

a major source of inoculum.

During the early part of the 1955-6 season a few more collections of trash were made from lands planted with tobacco in the preceding season. The presence of A. longipes was determined microscopically by methods (1) and (2) detailed above. With increasing rainfall, it became progesssively more difficult to find debris in these fields but some was found infested with the fungus as late as the beginning of February (Table IX). By this time, reaping of some plantings of tobacco in the district had commenced and Alternaria leaf-spot had been noticed and confirmed by examinations, on some plantings three weeks earlier.

TABLE IX

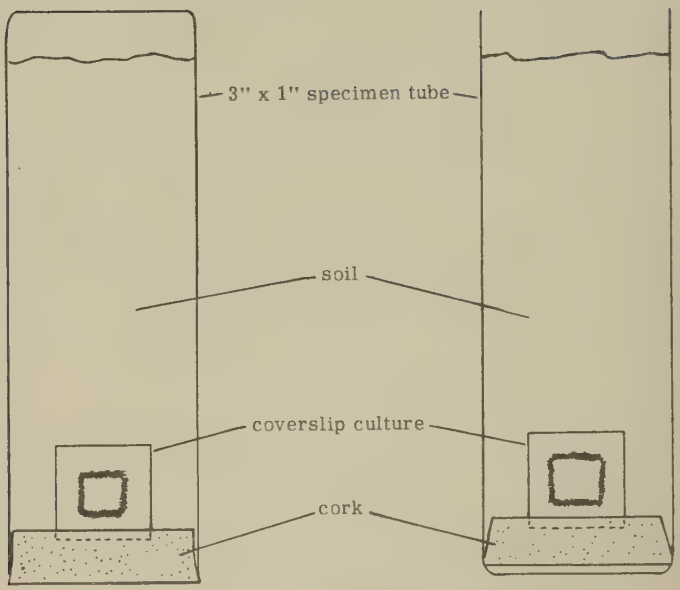
Collections of tobacco debris:
Kasungu district of Nyasaland, 1955-6

Date	Estate	Notes	A. longipes
23.12.55	Sable Creek	Stalks lying on surface of fields	+
9.1.56	Lisasadzi	Leaf trash in last year's land	+
10.1.56	Chingaliwa	Stalks lying in field	+
21.1.56	Sable Creek	Stalks protuding above soil	+
27.1.56	Kanjoka	Stalks lying in field; previously buried, uncovered when cultivating	+
1.2.56	Sable Creek	Stalks protruding above soil	+
3.2.56	Mpali	Stalks beneath grass cover	+

Survival of A. longipes in soil. In addition to survival on tobacco debris, the question arises whether A. longipes can exist independently in the soil as a saprophyte. Two small experiments were undertaken to investigate this.

Growth of A. longipes, spores and mycelium, was obtained on glass coverslips by means of Duncan slide cultures. Small pieces, approximately one sq. cm., were removed aseptically from a Petri dish poured with 2 per cent. potato-dextrose agar and transferred to a sterile slide, contained in a sterile dish. The sides of the agar block were then inoculated with spores of A. longipes and the block covered with a sterile cover-glass. These were incubated at 28°C. for seven days. At the end of this period, the agar had dried and the cover-glass with its ring of fungal growth was removed from the agar. These coverslips were then buried in sandveld soil collected from tobacco lands in the manner indicated in Figure 1 (a & b).

A number of tubes were set up, some with dry soil and others with moistened soil. The moisture content was maintained by daily additions of water, the tubes having been previously weighed. At intervals, coverslips were removed and the viability of the fungus tested. This was initially done by washing the coverslips in two changes of sterile water, transferring it to a sterile Petri dish, covering with maize-meal agar containing 2 per cent. potassium thiocyanate and incubating the poured plates at 28°C. The plates were then examined for growth of A. longipes. It was found, however, that the rapid growth of saprophytic fungi frequently made the determination of A. longipes difficult. Accordingly, coverslips removed from soil were washed in sterile water, clipped to a leaf on a 12-week old tobacco seedling and this incubated in a cabinet maintained at 75° to 80°F and 80 to 90 per cent. R.H. After approximately five days, a portion of the leaf under



(a) Experiment 1
closed tube

(b) Experiment 2
open tube

Fig. 1. Method for studying survival of A. longipes in soil.

the coverslip, whether visibly infected or not, was removed, washed in 0.01 per cent. mercuric chloride for two minutes, rinsed in sterile water, and plated on to maize-meal-potassium thiocyanate agar as before. It was found that using this method, most saprophytic fungi were eliminated and growth of A. longipes could be readily determined.

The results using the above methods are given in Table X.

TABLE X

Survival of A. longipes in sandveld soil

Time (days)	Experiment 1		Experiment 2	
	Dry soil	Moist soil	Dry soil	Moist soil
7*	+	+	+	+
9*	+	no test	no test	no test
14	+	-	+	+
21	+	-	+	-
29	+	-	-	-

* viability tests by direct plating, the other tests were preceded by screening through tobacco.

It appears that A. longipes can survive in dry soil independently of tobacco trash for at least one month, but that it survives in moist soil only for a period of about 7 to 14 days. It is possible that an increase in microbial activity in moist soil, compared with dry soil is the factor concerned. Direct microscopical examination of coverslips removed from dry soil showed no indication of new growth from spores or mycelium, the indications being that the fungus merely survives and does not grow in soil under these conditions. The evidence obtained suggests that if the fungus survived in soil during the dry season it would be rapidly eliminated at the onset of the rains. Carry-over in this way is unlikely, therefore, to be important in relation to the epidemiology of the disease.

Alternative hosts. It has been suggested that A. longipes can live on certain indigenous weeds and might in this way be carried over from one tobacco season to the next. Experiments by Tisdale and Wadkins (1931) and Riley (1949) give little support to this view. Three plants in particular have received special attention, Solanum panduraeforme, Nicandra physaloides, and Datura stramonium, since these are common weeds in Rhodesian tobacco growing areas which have been noted with lesions of the type caused by A. longipes (Riley, 1949). Of these, Solanum panduraeforme is of particular interest since it is very common in the Kasungu district on old tobacco lands and is frequently found to be infected by a species of Alternaria with spores similar to those of A. longipes. Another species of Solanum, S. incanum, which is common in the district, was found to be similarly infetced.

A number of small experiments were carried out to determine whether this Alternaria sp. could infect tobacco and whether A. longipes could infect either of the two Solanum spp. Small squares of leaf tissue from flue-cured tobacco (var. Delcrest) and from S. panduraeforme were placed in Petri dishes containing damp filter paper and inoculated by placing a small piece of infected tissue on them. Squares of leaf tissue inoculated with distilled water served as controls. These were incubated at room temperature for 5 to 7 days. The results are summarised in Table XI.

Some slight infection of S. panduraeforme by A. longipes was obtained, a result in agreement with that of Riley (1950), but no infection of tobacco with the Alternaria sp. from S. panduraeforme or with that from S. incanum could be obtained although under similar experimental conditions tobacco was rapidly infected by A. longipes. The results suggest that neither of these Alternaria spp. is A. longipes.

TABLE XI

Inoculation experiments with Alternaria longipes and Alternaria spp. from Solanum panduraeforme and S. incanum

Inoculated with	Flue-cured tobacco	Solanum panduraeforme
A. longipes	severe necrosis and yellowing	slight necrosis
Alternaria sp. from S. panduraeforme	no reaction	slight necrosis
Alternaria sp. from S. incanum	no reaction	slight necrosis
Control	no reaction	no reaction

It is possible that a wild host of A. longipes may yet be found but it is doubtful whether it would prove to be important as a source of inocolum. All the evidence to date indicates that tobacco debris is the main source of inoculum.

(b) Spore Dispersal

The conidia of A. longipes are borne in chains in great profusion over the surface of the lesion. These spores are mainly wind-borne but splashing raindrops may effect some local dispersal from the lesions to the leaves of the same or adjacent plants. During the 1955-6 tobacco season a study of spore dispersal was made using spore traps. Two types of trap were used, one designed at the Tobacco Research Board of Rhodesia and Nyasaland in conjunction with the Royal Rhodesian Air Force (referred to as the T.R.B. trap) and the other designed by Gregory, details of which have already been published (Gregory, 1954).*

The T.R.B. trap was used to obtain measurements of the number of spores per day at different sites. The figures obtained with this instrument are not strictly quantitative but are useful for comparative purposes.

In each test with the Gregory spore trap the same period for sampling was used throughout the test to allow a direct comparison to be made between the spore counts obtained.

The handle of the trap was also turned at a constant rate of 60 turns per minute for all measurements. This was necessary since a faulty altimeter prevented direct assessment of the air intake. At this speed the air intake would be approximately 9 l. per minute but since this is only an approximation the number of spores per cu. metre has not been calculated. When examining the slides all spores were counted on the trace.

Spore dispersal at different sites. It might be expected that, if tobacco debris is the main source of inoculum, there would initially be a greater spore load at sites where this is abundant. Five sites were chosen in the Kasungu district and a T.R.B. spore trap placed at each. The counts over a 20-day period from the 26th January to the 14th February, 1956, are given in Table XII.

Throughout this period very little Alternaria leaf-spot could be found in the tobacco at sites 2, 3, and 4. The highest spore counts were obtained at the site where debris from the previous season's crop had not been adequately destroyed.

* The author is indebted to the Tobacco Research Board for supplying the plans of the T.R.B. spore trap and to the Nyasaland Tobacco Association for a grant to pay for its manufacture, also to Professor P.H. Gregory for loaning his portable volumetric spore trap.

TABLE XII

Spore counts at different sites

Estate	Site	Total spore count
1	In the bush, $\frac{1}{4}$ ml. from tobacco fields	1
2	Nr. barns, and adjacent to tobacco field (reverted land)	16
3	In tobacco field (reverted land)	24
4	In tobacco field (second year land)	77
5	Abandoned field with regrowths and debris	622

A fairly high count was also obtained at site 4, which was a second year land where a reasonable attempt had been made to dispose of debris from the previous crop. Spore counts at the other sites were low, particularly where the trap was surrounded by bush. This again suggests that alternative indigenous host plants, if they exist at all, are unimportant as sources of inoculum.

Spore dispersal during the growth of a tobacco crop. One T.R.B. spore trap was set up in a tobacco field at the Tobacco Experiment Station, Lisasadzi, and kept there throughout the growing season in order to follow the changes in the spore content of the air in relation to the weather and growth of the tobacco. The result is presented in Figure 2. Roughly four periods of differing weather conditions may be distinguished with associated changes in spore counts. These are:

Period A. (6th December to 2nd January). There were initially some heavy showers of rain in this period followed by dry weather. Spores of A. longipes were found to be in the air on each day and on some days the counts indicated a relatively large spore load.

Period B. (3rd January to 15th February). This was a period of wet weather. Initially the showers were fairly heavy and of short duration but during the last three weeks most of the days were overcast with long periods of light rain and very little sunshine. Spore counts were high at first but diminished with the prolonged dull weather and on several days no spores were recorded.

Period C. (16th February to 17th March). This was a dry, sunny spell and is characterised by increasingly large spore counts.

Period D. (18th March to 19th April). This was a period of dry weather interspersed with heavy showers. The spore counts show corresponding fluctuations.

The overall picture obtained from these data is that dispersal of spores occurs during periods of dry weather, which would be expected with this type of fungus (Gregory, 1954). Alternaria leaf-spot was not observed in the field until the 2nd January. Since the land had not been planted with tobacco in the previous season and there was, therefore, no debris, the spores noted in counts prior to this must have come from external sources. This could have been from debris in an adjacent field planted with tobacco in the previous season but very little debris could be found there and it is more likely that the spores came from a field approximately a mile away which had a considerable amount of infested debris and a number of infected regrowths. The main period of reaping took place at the end of a rainy period (B) and there was little development of the disease within the crop during this period. The amount of Alternaria leaf-spot increased rapidly, however, on the top leaves and these were badly spotted when reaped. This increase was associated with a relatively dry spell of weather and the spore counts suggest that more favourable conditions for spore dispersal prevailed at the time. Reaping

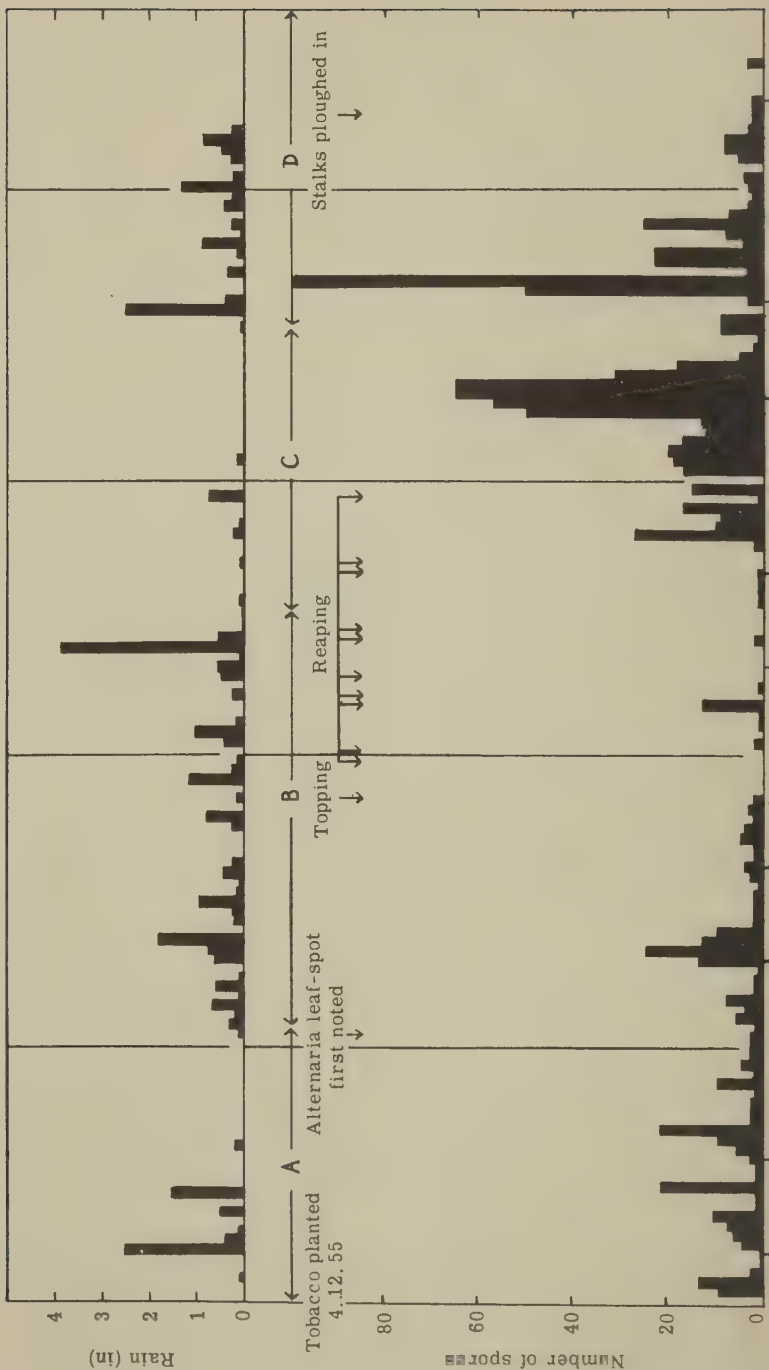


Fig. 2. Spore dispersal during the growth of a tobacco crop.

finished on the 28th February and the stalks were cut to within six inches of the ground to facilitate ploughing. This was delayed, however, and in the intervening period the stalks and suckers became increasingly infected with Alternaria leaf-spot and increasingly high spore counts were registered. By thus delaying ploughing-in, the debris became potentially more dangerous as a source of inoculum for the next season.

Spore dispersal in relation to weather conditions. The above data give some idea of the relationship between spore dispersal and weather conditions, but these lack precision in so far as the counts obtained are not quantitative measurements of the spore load of the air and give only an overall assessment for a 24 hour period. More precise measurements of the fluctuations in spore load relative to the weather conditions were obtained using the Gregory portable spore trap, which is an intrsument capable of quantitative measurements over short periods. A number of measurements were taken at two sites and these are given in detail below.

Test 1. (1st - 2nd March, 1956). Trap placed 30 yds. from plot of tobacco grown on second year land and affected by Alternaria leaf-spot. Eleven sampling periods of 5 minutes were taken with 1 minute intervals, for each reading. The preceding day had been generally fine and sunny with a trace of rain recorded.

Time	Weather conditions	No. of spores
7.00-8.00	Sunny with little cloud. Light breeze. Heavy dew on ground	5
11.00-12.00	Sunny but with increasing cloud. Light breeze. Dry.	5
15.00-16.00	Dull. Heavy cloud. High wind preceding r ain which came at the end of sampling period. Rainfall 0.72 in.	70
19.00-20.00	Sky clear with little cloud. No wind. Ground very wet.	8
23.00-24.00	Sky clear. No cloud or wind. Ground very wet.	0
7.00-8.00	Cloudy. Gusts of wind. Ground very wet.	1
11.00-12.00	Sunny. Strong wind. Dry	2539
15.00-16.00	Sunny. Gusts of wind. Dry.	3

Test 2. (13th to 16th March, 1956). Trap placed on contour ridge within a field of tobacco affected by Alternaria leaf-spot and grown on reverted land. Five sampling periods of 5 minutes were taken with 1 minute intervals, for each reading. The preceding day had been sunny with no rain.

Time	Weather conditions	No. of spores
9.15-9.45	Cloudy with short intervals of sunshine. Gusts of wind. Light rain (not enough to record).	2
10.15-10.45	Cloudy and windy. Dry.	5
11.15-11.45	Cloudy with some sunshine. Windy.	7
12.15-12.45	-do-	1
13.15-13.45	Sunny and windy.	1

14.15-14.45	Sunny. Very windy.	0
15.15-15.45	Sunny. No wind.	0
16.15-16.45	Sunny. Very windy.	2
17.15-17.45	Cloudy and windy.	0

Shortly after sunset a heavy mist arose and remained until the morning of the 14th.

9.15-9.45	Sunny. Gusts of wind. Ground damp but drying rapidly.	70
10.45-10.45	Sunny. Gusts of wind. Dry.	1384
11.15-11.45	-do-	1784
12.15-12.45	Sunny but with increasing cloud. Windy. Dry.	0
13.15-13.45	-do-	11
14.15-14.45	-do-	13
15.15-15.45	Cloudy. Occasional gusts of wind.	0
16.15-16.45	-do-	0
17.15-17.45	Cloudy. No wind. Dry. The night was clear and dry.	0
9.15-9.45	Cloud. Little wind. Dry.	4
10.15-10.45	Cloudy. No wind. Slight drizzle.	4
11.15-11.45	Cloudy. No wind. Dry.	0
12.15-12.45	Sunny. Windy. Dry.	4
13.15-13.45	-do-	0
14.15-14.45	Cloudy. Slight drizzle.	4

The weather cleared towards sunset and the night was dry. There was a little early morning dew.

7.30-8.00	Sunny. Light wind. Ground slightly wet.	0
9.00-9.30	Sunny. No wind. Dry.	2
10.00-10.30	-do-	1
11.00-11.30	Sunny. Light wind. Dry.	5
12.00-12.30	-do-	1
13.00-13.30	Sunny. No wind. Dry.	1

The results confirm that spore dispersal only takes place during periods of dry, sunny weather. They indicate also, however, that this must be preceded by a period of high humidity which is presumably necessary for spore production.

Alternating periods of rain and sun, particularly warm, damp nights followed by dry, sunny days appear generally to be most favourable for spore production

and dispersal. Such conditions frequently occur during the tobacco season in Nyasaland. The spore counts obtained indicate that at times the spore load of the air may be considerable. Counts of over 250 spores per cu. ft. have been recorded, but these are only observed over short periods, usually at mid-morning on days when conditions are favourable for dispersal.

(c) Infection and Disease development

(i) Seedling infection

Alternaria leaf-spot is typically a disease of mature tobacco. Lesions are first noted in the field on the lugs some 2-3 weeks before ripening. Seedlings are rarely found to be infected but a few instances have been recorded (Hopkins, 1948). Riley (1949) found that tobacco seedlings could be infected by artificial inoculation but the symptoms produced were atypical and the percentage infection generally low. Susceptibility of seedlings did, however, increase with age. A low percentage germination of spores on seedling leaves was found to be partly responsible for low percentage infection and further investigation (Riley, 1950) indicated that the high number of hairs on seedling leaves might, in some way, be responsible for this.

During the present investigation of the disease a number of experiments were carried out on seedling infection similar to those of Riley. Seedlings (var. Delcrest) of various ages were obtained from seedbeds at Kutsaga Station, Tobacco Research Board of Rhodesia and Nyasaland. Other seedlings were raised in the laboratory in waxed cartons containing washed sand. These were watered once a week with a x20 dilution of Steinberg's medium (Steinberg, 1947) and the remainder of the time with distilled water when necessary.

Inoculations were carried out by spraying leaves with a spore suspension of A. longipes, using an atomiser. The culture of A. longipes was isolated from infected tobacco and was shown to be strongly pathogenic in tests carried out on mature leaves.

An examination of spore germination on seedling leaves was made in two ways, either by clearing and mounting small portions of tissue in lactophenol or by using the celloidin strip method described by Wenzl (1939). These strips were mounted and stained in cotton blue and lactophenol to facilitate counting.

Infection of six-week old seedlings. Six-week old seedlings were obtained from the seedbeds and transferred to 3-inch pots. 19 of these were inoculated with A. longipes and a similar number treated with distilled water. The plants were then transferred to a closed cabinet (at approximately 70°F and 90-95 per cent. R.H.) under illumination. In five days, inoculated plants showed small, brown, slightly sunken lesions of $\frac{1}{2}$-1 mm. diameter. These lesions developed most markedly on the lower leaves and in some instances were associated with intense yellowing. Microscopical examination of a number of lesions showed germination of spores and penetration of the epidermal cells. In most instances the cuticle was directly penetrated but some examples of stomatal penetration were observed. All seedlings inoculated with the spore suspension developed lesions. No reaction was observed on the controls. At the end of 10 days the plants were transferred to the greenhouse for observations on the development of the disease. The lesions showed only local development on the leaves. The lower leaves which were infected died off and no infection was observed on new growth.

Age of seedling in relation to spore germination and infection. Seedlings of ages ranging from three to eleven weeks, raised in the laboratory, were divided into two series, one inoculated with A. longipes, the other similarly treated with distilled water. For comparison, six 12-week old seedlings were obtained from the seedbeds, three inoculated with A. longipes and three with distilled water in the same way. All the plants were then placed in a cabinet maintained at 30 ± 2°C and 80-90 per cent. R.H. At the end of 5 day's incubation, small brown, necrotic lesions had developed on those seedlings obtained from the seedbeds and inoculated with A. longipes.

These lesions later increased in size and developed a marked yellow halo. Isolations were made from these seedlings by washing pieces of infected leaf tissue in 0.01 per cent. mercuric chloride for two minutes, washing in two changes of sterile water and plating on to maize-meal agar containing 2 per cent. potassium thiocyanate. A. longipes was re-isolated from 6 of a total of 10 pieces of tissue plated out.

No infection was observed in any of the plants grown in the laboratory, which were sprayed with spores of A. longipes. In order to ascertain whether this was owing to lack of spore germination, a count was carried out by direct microscopical examination of portions of cleared leaf. The result is given in Table XIII.

TABLE XIII

Spore germination on seedling leaves of varying ages
(seedlings grown in the laboratory)

Age of seedling	No. of spores counted	Percentage germiantion	
11 weeks	100	49)	
	100	49)	49
	100	50)	
7 weeks	100	36)	
	100	31)	32
	100	29)	
3 weeks	100	17)	
	100	20)	19
	100	20)	

It is apparent that lack of visible infection in these seedlings was not due to lack of spore germination. In addition, however, no penetration was observed in any of the material examined, so that the possibility of a dormant infection can be discounted

The relationship between age of seedling and percentage germination observed (Table XIII) is striking. This was investigated further in the following experiment. Leaves were taken from seedlings of various ages grown in the seedbeds, placed in Petri dishes containing damp filter paper and inoculated with A. longipes. Small squares of cellophane in Petri dishes were similarly treated for comparison. Spore germination counts were carried out using the celloidin strip method. Counts were made after 16 and 41 hours incubation and are recorded in Table XIV.

TABLE XIV

Spore germination on seedling leaves of varying ages
(seedlings grown in seedbeds)

Age of seedling	Percentage germination	
	16 hrs. incubation	41 hrs. incubation
12 weeks	68.7	84.0
7 weeks	52.7	54.7
5 weeks	52.0	52.0
Cellophane	82.6	84.0

The results indicate a marked difference in spore germination on leaves from 12-week old seedlings as compared with that from 7- and 5-week seedlings. Spore

germination on leaves taken from 12-week old seedlings was comparable with that on cellophane after 41 hours but germination on cellophane was more rapid. There was no difference in the spore germination on leaves from 7-week old seedlings and that on leaves from 5-week old seedlings. The percentage germination was generally higher than that obtained in the preceding experiment, but since different batches of spore suspension were used, no conclusions can be drawn from this.

Type of seedling leaf in relation to spore germination. It was observed in the above experiments where seedlings were infected that lesions developed almost exclusively on the lower leaves and not on the top leaves. This, coupled with the relationship observed between spore germination and age of seedling, suggested that there might be differences in spore germination on leaves taken at different levels from seedlings of the same age. Samples of yellowing bottom leaves, middle leaves and young top leaves were therefore taken from 12-week old seedlings grown in the seedbeds. These were placed in Petri dishes lined with damp filter paper, inoculated with A. longipes and incubated at 28°C as before. Spore germination counts were carried out after 21, 43 and 67 hours incubation. The counts at 67 hours are given in Table XV.

TABLE XV

Spore germination on three types of seedling leaf

Type of leaf	No. of spores counted	No. germinated	Percentage germination
Yellow, bottom leaf	181	167	92.3
Middle leaf	136	126	92.7
Top leaf	233	203	87.1

It can be seen that there were no marked differences in the percentage spore germination on the three types of leaf.

The effect of the removal of leaf hairs on spore germination. Riley (1950) found that removal of the hairs from seedling leaves by scraping and singeing resulted in increased infection. The effect on spore germination of removing the hairs from seedling leaves was examined in the following experiment. Leaves were taken from 15-week old seedlings grown in the seedbeds. One half of each leaf was covered with celloidin and the dried celloidin subsequently stripped off. In this way most of the hairs on one side of the leaf were removed. The leaves were then inoculated with A. longipes and incubated at 28°C. in Petri dishes containing damp filter paper. After 19 hours' incubation, a spore germination count was carried out, using the celloidin strip method, and measurements of germ-tube length taken on both normal and striped leaf. The results (Table XVI) show that not only was spore germination greater on leaves where the hairs had been removed, but also there was more vigorous growth of the germ-tubes.

TABLE XVI

The effect on spore germination of removing the hairs from seedling leaves

Leaf	No. of spores counted	Percentage germination	Length of germ-tube*
Normal	50	72)	
	50	74) 72.7	50μ
	50	72)	
Leaf with hairs removed	50	92)	
	50	86) 89.3	210μ
	50	90)	

* mean of 25 measurements

It seems clear from the above experiments and from those of Riley (1949, 1950) that seedlings can become infected with A. longipes. Repeated examination of lesions on seedling leaves taken from seedbeds failed, however, to show the presence of A. longipes. The atypical symptoms produced in seedlings, however, does make diagnosis in the seedbeds difficult and possibly some transfer of the disease to the lands may occur by this means. This could be important in establishing the disease within a crop grown on a newly opened land but in general it seems unlikely that seedling infection is important in relation to the spread of the disease in the lands.

(ii) Development of the disease within the ripening crop

Alternaria leaf-spot seldom develops rapidly before the bottom leaves are harvested. Once the plant has been topped, however, and the lugs ripen the disease may, under favourable conditions, spread very rapidly throughout the crop. The progress of the disease was followed closely on two plantings on one estate in the Kasungu district during the 1954-55 season. (Fig. 3).

The investigation was started on the 23rd February, 1955. By this time, there had already been epiphytotics of the disease on tobacco in fields 1 and 2, and there were lesions on the lower leaves of the tobacco in Field 3. The disease subsequently became epiphytotic in this field. These fields (Nos. 1, 2 &3) were previously planted with tobacco in the 1953-54 season. The remaining two fields (Nos. 4 & 5), in which the observations were carried out, were in their first year of cultivation. The land had been stumped and cleared during the dry season, 1954. Details of the fertiliser treatments and cultural operations for these two fields are given in Table XVII.

TABLE XVII

Fertiliser treatments and cultural operations, Fields 4 & 5

Field No.	4	5
Size:	8 acres	8 acres
Tobacco variety:	Yellow Mammoth	Delcrest
Fertiliser:	Pre-fertilised with 475 lb. "V" mixture (4.18.15) plus top-dressing 75 lb. "E" mixture (8.0.16) within 3 weeks of planting i.e. 25 lb./ac. N $85\frac{1}{2}$ lb./ac. P_2O_5 79 lb./ac. K_2O	Pre-fertilised with 375 lb. "V" plus top-dressing 100 lb. "V" and 75 lb. "E" within 3 weeks of planting, i.e., 25 lb./ac. N $85\frac{1}{2}$ lb./ac. P_2O_5 79 lb./ac. K_2O
Dates of planting:	8.1.55	7.1.55
Priming:	24.2.55	No priming
Topping:	24.2.55	24.2.55
Reaping:	26.2.55 3.3.55 5.3.55 9.3.55 15.3.55 19.3.55 24.3.55 31.3.55	3.3.55 5.3.55 11.3.55 16.3.55 21.3.55 26.3.55 31.3.55 Plus two more reapings between this last date and April 10th.

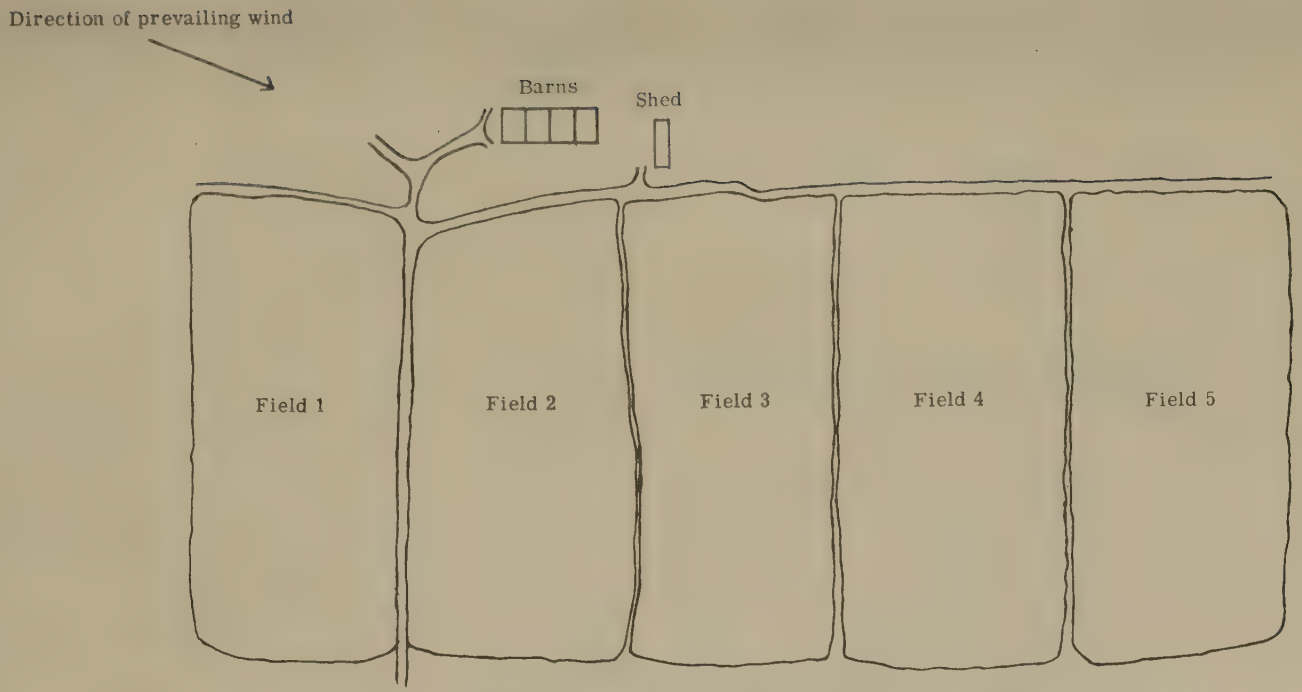


Fig. 3. Sketch map: Layout of fields, Sable Creek Farm.

The amount of the disease was assessed by counting lesions on plants in groups of ten randomised throughout the field. Towards the end of the reapings when the number of lesions per leaf was considerable an assessment was made to the nearest ten lesions per leaf. No count was possible on leaves with large areas of necrosed tissue resulting from the coalescing of numerous lesions and these were given a score of 100. The number of lesions on reaped leaves was counted so that a complete record of lesion development was obtained for each plant.

The development of the disease within the crop on field 4 occurred in three stages. A few lesions were noted on the lugs of some plants on the 24th February. By the 1st-2nd March infection of the lower leaves was general throughout the crop. Disease development entered its second stage following reapings on the 3rd and 5th March. There were rapid increases in the number of lesions during this period; prominent lesions could be found on the cutters and middle leaf and pin-point lesions on the tips. During this phase the disease became epiphytotic. The third stage in disease development started about the 13th-14th March. During this period there was no appreciable rainfall but there were heavy morning dews which left the leaves very wet for the first hour or so after sunrise. These conditions were apparently favourable for spore germination and a considerable number of secondary lesions developed around lesions which were already sporulating. Subsequently these lesions coalesced and resulted in large areas of necrosed tissue. In this phase, the disease did most damage.

Disease development within the tobacco on field 5 also fell into three fairly well defined stages but the incidence of the disease was considerably lower. A few lesions were again noted on the 24th February. By the 4th March the lower leaves throughout the crop were infected. There was a marked increase in the number of lesions following the reaping on the 5th March but there was less spread of the disease up the plant than there was in field 4. Prominent lesions could be found on the cutters, a few pin-point lesions on the middle leaves but none on the tips. A certain amount of secondary lesion development occurred but this was limited, there being fewer lesions originally, and only a small number of leaves became so necrosed as to be valueless.

Normal reaping practices as carried out on this crop failed to check the development of the disease. The number of lesions increased following each reaping often to a figure greater than before reaping. (Figure 4) Disease incidence appeared to be closely related to the ripening of the leaves. Increases in total lesions counts were paralleled by increases in the total number of ripe leaves (Figure 5). Increase in lesion development following reaping may have been due to the ripening of more leaves on the plant associated with an increased susceptibility. It is possible also that the movement of the reapers through the field and the mechanical operation of reaping helped to disseminate the spores of the fungus.

The difference in the incidence of the disease within the two plantings of tobacco was striking. The fact that different tobacco varieties were involved does not provide sufficient reason for this difference since information regarding disease incidence in the district indicated that there were no marked differences in the susceptibility of Yellow Mammoth and Delcrest varieties to infection by A. longipes. The proximity of infected tobacco in fields 1, 2 and 3 makes it unlikely that the size of inoculum was the limiting factor in disease development within field 5, and the similarity in fertiliser application and cultural operations on the two plantings makes it difficult to relate differences in disease incidence directly to these factors. There was a difference, however, in the ripening of the two plantings and this, in relation to rainfall, may account for the difference observed.

The basic requirements for disease development are the presence of inoculum (spores), the presence of susceptible host tissue and suitable conditions for spore germination and penetration. During the period from the 20th February to the 7th March these requirements were apparently met and the disease developed in both plantings. It may be argued, however, that whereas tobacco in field 4 was maturing rapidly during this period (as indicated by the reapings) tobacco in field 5 was not, and that it was the difference in quantity of ripe or nearly ripe leaf which determined the degree of infection and subsequent difference in disease incidence.

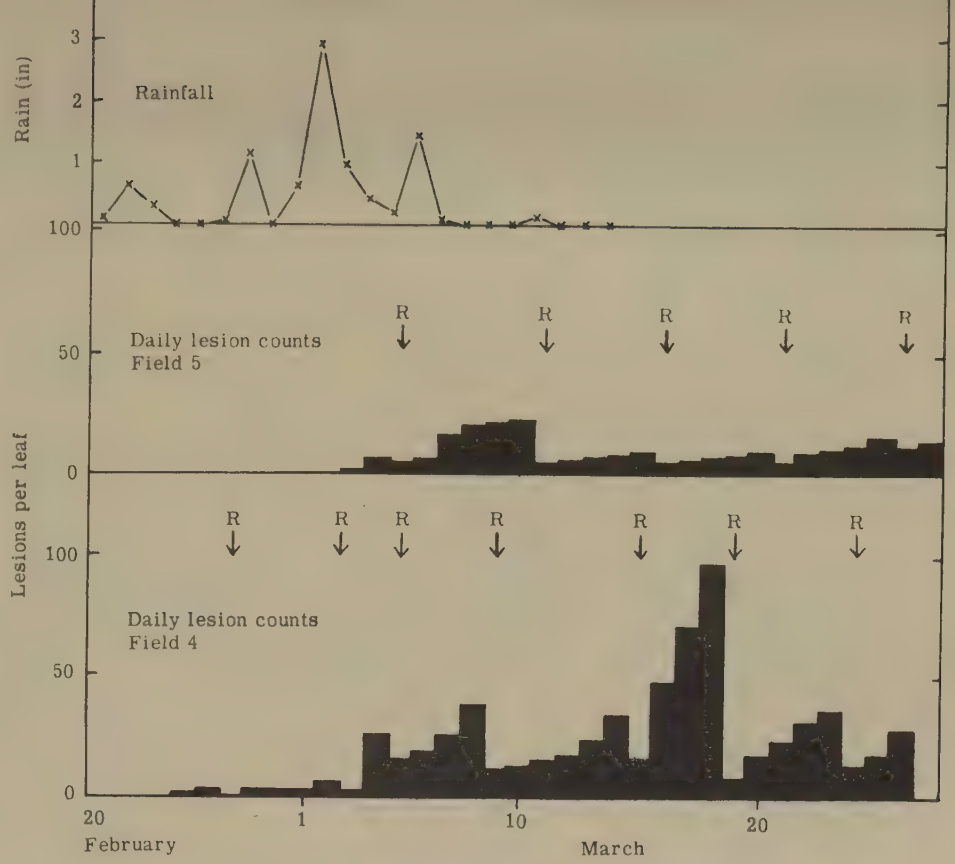


Fig. 4. Disease development in relation to reaping.

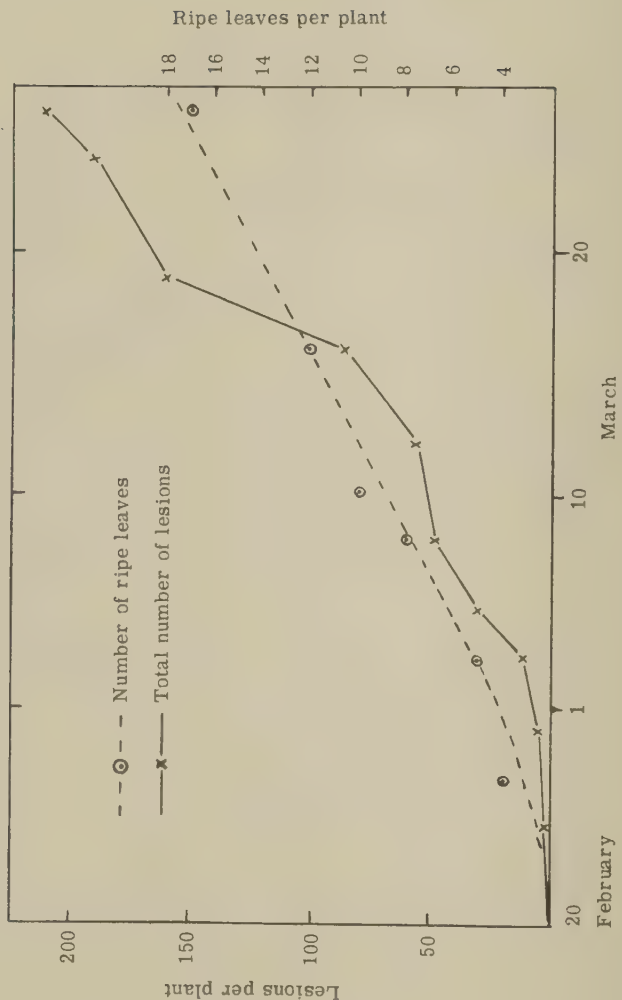


Fig. 5. Disease incidence in relation to ripening.

However, some caution is needed in interpreting too rigidly results which were obtained from observations and not by direct experimental methods.

(iii) Some factors affecting the development and severity of the disease

Fertilisation and cultural operations such as topping and priming have marked effects on the growth of a tobacco crop and because of this, it is likely that they will also have an effect on the severity of Alternaria leaf-spot. There were indications from the survey of the Kasungu district that fertilisation was, in fact, one of the more important factors influencing the severity of the disease. Further information on the effect of certain cultural practices on disease incidence was obtained from field experiments conducted in the Kasungu district during the 1954-5 and 1955-6 tobacco seasons.

Fertilisation The effect of nitrogen, phosphate, and potash on disease incidence was examined in two field experiments during the 1955-6 season. The first of these was sited on reverted land and consisted of 2^3 factorial with the following levels of N, P and K: 12 and 24 lbs. N per acre, nil and 100 lbs. P_2O_5 per acre and nil and 90 lbs. K_2O per acre. The eight fertiliser mixtures involved were made up using ammonium sulphate, superphosphate and potassium sulphate and applied by hand in a ring around the base of each plant. Disease assessments were carried out at intervals by counting the number of lesions on 100 leaves taken from each plot at harvesting. All the leaves reaped (excluding those from guard rows) were bulked and the first hundred leaves examined. An analysis of the result is presented in Table XVIII.

TABLE XVIII

The effect of nitrogen, phosphate and potash on the incidence of Alternaria leaf-spot (reverted land).

	Treatment	Total lesion count	Effect	
(1)	12:0:0	5863	47047	Total
n	24:0:0	8738	+ 5335***	N
p	12:100:0	6550	- 4803***	P
np	24:100:0	7452	- 2151**	NP
k	12:0:90	5228	-10159***	K
nk	24:0:90	6096	- 2219**	NK
pk	12:100:90	3215	- 3605***	PK
npk	24:100:90	3905	+ 1795*	NPK

Significant levels:

*	.05	1440
**	.01	1998
***	.001	2779

The three main effects of N, P and K are highly significant. Addition of nitrogen results in an increase in lesion counts whereas phosphate and potash both markedly reduce lesion counts. Potash appears to exert a greater effect than phosphate in this respect. The effect of phosphate in reducing lesion counts is significantly greater at the higher rate of nitrogen than at the lower rate. The same is true of potash. It appears, in this instance, that the effect of nitrogen in increasing the number of lesions is rapidly reduced by a simultaneous increase in the phosphate or potash level. The results also show that the response to phosphate is greater in the presence of potash than in its absence.

The second fertiliser experiment was sited on second year land and consisted of the following levels of N, P and K: 12 and 24 lbs. N per acre, 36 and 108 lbs. P_2O_5 per acre and 30 and 90 lbs. K_2O per acre. Fertiliser application and disease assessments were carried out by similar methods to those described above. The results (Table XIX) again show that the effect of nitrogen is to increase the number of lesions whereas that of phosphate and potash is to decrease lesion counts.

In this experiment, the interactions (NP, NK, PK) were not significant.

TABLE XIX

The effect of nitrogen, phosphate and potash on the incidence of Alternaria leaf-spot (second year land)

Treatment		Total lesion count	Effect
(1)	12:36:30	6943	42783 Total
n	24:36:30	7388	+4125* N
p	12:108:30	4639	-7331** P
np	24:108:30	7351	-45 NP
k	12:36:90	4543	-9859** K
nk	24:36:90	6183	-2189 NK
pk	12:108:90	3204	-2649 PK
npk	24:108:90	2532	Confounded with blocks

Significant levels:
* .05 3586
**.01 5000

The results of these experiments are in general agreement with those obtained previously by Riley (1949) using sand cultures. He did not, however, in subsequent experiments, obtain the same responses to high phosphate and potash when these were added to soil, but the levels of P and K were lower than those used in the experiments described above.

Topping and priming. The effect of topping and priming on the incidence of Alternaria leaf-spot was examined in two field experiments. In each experiment the 12 treatments resulting from the combination of the following,

high topping
standard topping X priming X early reaping
low topping no priming normal reaping

were randomised in four blocks. Early reaping consisted of removing all leaves with Alternaria lesions irrespective of ripeness. It was carried out for the first two reapings only. Topping and priming (where applicable) was carried out on all plots at the same time. The number of leaves on each plant of each plot was also counted (excluding leaves "primed off" and corresponding leaves on "unprimed" plants). The final lesion counts were calculated on a leaf basis to compensate for the differences in leaf number resulting from the different topping treatments.

TABLE XX

Effect of height of topping on lesion development.

Height of topping	Lesion counts	
	Experiment 1	Experiment 2
High	179	66
Standard	233	77
Low	300	93
L.S.D. (.05)	89	12

An analysis of the results (Table XX) shows that in both experiments the height of topping had a significant effect on the lesion count. Plants topped low had significantly more lesions than plants topped at standard height or plants topped high. There was no significant difference, however, between the latter two treatments with regard to lesion count.

The priming/no priming and early reaping/normal reaping treatments showed no significant effect on lesion count. Low topping in these experiments resulted in the production of coarse, heavy leaves and it was probably this adverse effect on the nutrition of the plant which resulted in the high disease incidence observed.

(d) Field experiments with fungicides.

The possibility of controlling Alternaria leaf-spot by the use of fungicides, while presenting considerable difficulties, was investigated as thoroughly as possible. Some field trials were conducted during the 1954-5 season and further trials during the 1955-6 season.

(i) 1954-5 season

Dusting with a 50 per cent. copper dust. Two similar experiments were carried out within two plantings of tobacco. For each experiment, twelve plots of ten plants in four blocks were selected and the following three treatments:

no dusting (controls)
daily application of dust*
application of dust at 3-day intervals

randomised in these blocks giving four replicates of each treatment. The dust was applied in the early mornings, using a Craven "Rotary" hand duster. The application rate was approximately 100 lb. per acre. Disease assessments were made by lesion counts and the final total lesion counts for each treatment are given in Table XXI.

TABLE XXI

Effect of copper dusting on lesion development

Treatment	Lesion count	
	Experiment 1	Experiment 2
No dusting	8360	3294
Daily dusting	2677	2060
3-day dusting	2509	1648
L.S.D. (0.5)	1729	882

The results show that dusted plants were significantly less infected than non-dusted plants, but there was no significant difference between the two dusting treatments used. As a practical method of control, however, the dusting as carried out in the experiments was not satisfactory. During the heavy rains it was difficult to maintain an effective cover of fungicides on the plants. When the rains slackened off, a good cover of dust was obtained but this remained on the leaf until reaping. Examination of cured leaf from these plots showed that a considerable amount of dust remained, which would be unacceptable to the buyers.

* Applications of dust were, in fact, not possible every day because of the heavy rains.

(ii) 1955-6 season

Spraying with zineb and copper oxychloride. Two fungicides were used, one containing 65 per cent. zineb (zinc ethylene bisdithiocarbamate) and the other 50 per cent. oxychloride. These were applied at two rates:

1 & 2 lb. per 100 gallons for zineb, &

$2\frac{1}{2}$ & 5 lb. per 100 gallons for the copper oxychloride, using a knapsack pressure sprayer*

Three times of application were used for each spray concentration:

(i) from planting out, at 5-day intervals

(ii) spraying after each reaping

(iii) once only after the first reaping

A randomised block design was used giving four replicates of each individual spray treatment, together with non-sprayed (control) plots. The amount of disease was assessed by a grading system on the cutters, middle and top leaves. These were scored as follows:

0 no Alternaria leaf-spot

1 slight Alternaria leaf-spot

2 moderate Alternaria leaf-spot

3 severe Alternaria leaf-spot

and a final score for the plant obtained by addition. The results are presented in Table XXII.

TABLE XXII

The effect of spraying with zineb and copper oxychloride on the incidence of Alternaria leaf-spot

Fungicide:	65 per cent. zineb			50 per cent. copper oxychloride		
Rate per 100 gallons	1 lb.		2 lb.	$2\frac{1}{2}$ lb.		5 lb.
Time of application (see text)	(i)	(ii)	(iii)	(i)	(ii)	(iii)	(i)	(ii)	(iii)
Total disease assessment (control: 127)	112	191	153	103	104	113	127	105	101	108	173	63

No significant reduction in disease incidence was achieved by the spraying treatments. These treatments were carried out during a period of frequent rain and it was practically impossible to maintain an adequate cover on the leaves.

Spraying with zineb. A second experiment was carried out using a 65 per cent. zineb preparation at a rate of 2 lb. per 100 gallons. This was again applied using a knapsack pressure sprayer. There were three treatments:

* "Mystifier" sprayer (Drake & Fletcher, Ltd.)

(i) no spraying

(ii) spraying from planting-out at 5-day intervals

(iii) spraying from first observable infection, at 5-day intervals

A randomised block design was again used, giving four replicates of each treatment. Disease assessments were made by lesion counts. The result is presented in Table XXIII.

TABLE XXIII

Spraying with a 65 per cent. zineb preparation

Treatment	Lesion counts				
	Block I	Block II	Block III	Block IV	Totals
Nil	2 793	2439	3294	953	9479
from planting out	1 944	4243	2747	822	9756
from first infection	2 682	3510	1752	1196	9140

Again there was no reduction of the disease resulting from the spraying treatments and failure to maintain an adequate cover of fungicide on the leaves during the frequent rains is probably responsible for this.

IV DISCUSSION

The results obtained emphasize that Alternaria leaf-spot is a disease of the maturing leaf. While seedlings can be inoculated artificially there is no evidence to show that infection in the seed beds occurs to an extent where it might be important in relation to the spread of the disease in the lands. It must be admitted, however, that the atypical symptoms produced in seedlings makes diagnosis of the disease in the seed beds difficult. The mechanism of seedling resistance is an interesting problem as the leaf hairs appear to affect spore germination in some way. There are indications, however, particularly in the relationship between age and susceptibility, that internal chemical changes in the plant may be the predominating factor controlling infection. This is supported by observations on the development of the disease in the field.

It has been suggested that A. longipes may infect young plants and produce a dormant infection without visible lesion development. This hypothesis was suggested by the relatively long period between the development of the spores on tobacco debris, on which the fungus overwinters, and the appearance of the disease in the new tobacco crop. Studies on spore dispersal have shown, however, that there is no lack of inoculum from these sources even as late as January-February when the tobacco begins to ripen and becomes susceptible. The possibility of dormant infections can not be entirely discounted but it is not necessary in order to explain the epidemiology of the disease.

The fact that infested tobacco debris appears to be the main source of inoculum suggests that one way of reducing disease incidence would be to remove as much of this debris as possible. Observations in the Kasungu district indicate that field sanitation is indeed important if the disease is to be kept at a low level. Two methods have generally been used for the disposal of the trash, burning and burying. The danger of the former method is that the debris must be left for a considerable period to dry before burning and during this period innumerable small pieces of infested leaf tissue are blown over the field and into the surrounding bush. A considerable portion of the debris, therefore, is not destroyed. In addition, with

burning, there is a loss of plant material which could have provided organic matter for the soil.

The alternative of burying the debris is to be preferred but it is not without its difficulties. Ideally, the stalks and other trash should be ploughed in as soon as reaping has finished and the field harrowed later. Very good results were obtained where this was done and virtually no trash could be found early in the next tobacco season. Some farmers, however, have not the machinery and rely solely on native labour. Here the best that can be done is to remove the stalks, lay them between the ridges and hoe the soil over them. Unfortunately, the rains are nearly finishing by the time material is buried and little decomposition takes place until the commencement of the next rains. Although decomposition may then be rapid there is inevitably a period when infested material is present and a potential danger to the new crop, if exposed. This danger is greater where the stalks have been buried by hand since they are not buried deeply and wind erosion frequently exposes them again during the dry season.

The planting of the same land in the following season is quite inadvisable since ridging operations will further expose buried debris. There is also some danger of regrowth where stalks have been buried, but close supervision of land should ensure that these are destroyed immediately.

Fertilisation of the tobacco crop can also have a marked effect on subsequent disease incidence. Not only has this been established in field experiments but also there are indications from the survey of the Kasnugu district that modification of fertilisation practices has been largely responsible for the decline in disease incidence over the past few years.

Attempts to control the disease by the use of fungicides were disappointing. In two experiments, some measure of control was achieved which can be attributed to a period of dry weather intevening when an effective cover of fungicide could be maintained on the plants. There seems little doubt that many fungicides do prevent growth of the fungus but the difficulties of spraying or dusting during the rains when tobacco is grown, are such that these methods cannot be offered as of practical use.

Information collected during these investigations suggests, in fact, that the control of the disease depends on two things, the eradication of the pathogen wherever possible and the use of those cultural practices which experience and experiment show to be most beneficial for the nutrition of the tobacco plant. In the Kasungu district there was a marked decline in the incidence of Alternaria leaf-spot during the years 1952-6. This was associated with changes in cultural practices and with an increasing awareness on the part of the growers for the necessity of adequate field sanitation.

V SUMMARY

1. Information collected from a disease survey in the Kasungu district of Nyasaland showed that:

(a) Alternaria leaf-spot was more prevalent on tobacco planted on second year land and on heavy (sandy clay loam) soils.

(b) A higher proportion of tobacco plantings which received over 24 lbs. nitrogen per acre were severely infected with Alternaria leaf-spot than those which received lower rates. Similarly, more plantings which received low amounts of phosphate and potash (less than 100 lbs. P_2O_5 and/or 90 lbs. K_2O per acre) were severely infected than those which received higher amounts.

(c) A higher proportion of plantings which received additional fertiliser as side-dressings were severely infected than those which received no side-dressings.

2. The fungus, Alternaria longipes (Ell. & Ev.) Mason, which causes Alternaria leaf-spot of tobacco, can survive through the dry season in Nyasaland on tobacco debris. This appears to be the main source of inoculum.

3. The fungus can live in dry soil independently of tobacco trash for a month or longer but exists in moist soil for only 7 to 14 days. Carry-over in soil is thus not likely to be important.

4. The existence of alternative hosts amongst indigenous plants is doubtful and also unlikely to be important as a source of inoculum.

5. Dry, sunny conditions are most favourable for spore dispersal but these must be preceded by periods of high humidity which are favourable for spore production.

6. Experiments on seedling infection showed that:
(a) Six-week old tobacco seedlings could be infected with Alternaria longipes but that the symptoms produced were not typical of the disease as seen in the field.
(b) Spore germination on seedling leaves varied with the age of the seedling but no differences were observed on leaves taken at different levels from seedlings of the same age.
(c) Removal of the hairs from seedling leaves resulted in an increase in spore germination and in the growth of the germ-tubes. Seedling infection appears to be of little or no importance in the nurseries.

7. Alternaria leaf-spot can rarely be detected within a crop earlier than 2-3 weeks before the lugs ripen. Development of the disease is usually slow until reaping starts when, under favourable conditions, it may become epiphytotic within a week. Susceptibility of the leaves seems to increase with ripening.

8. It was confirmed in field experiments that nitrogen increases and phosphate and potash decrease the susceptibility of tobacco to infection.

9. In two experiments on topping and priming disease incidence was greater on plants topped low than those topped to standard height or topped high. This was associated with the production of heavy, coarse leaves on plants topped low. Priming treatments had no significant effect on disease incidence.

10. In field trials with certain dusts and sprays some measure of control was obtained in some experiments but not in others. Failure to obtain control is attributed to the difficulty in maintaining an adequate cover of fungicide on the leaves during the frequent rains. Spraying or dusting is not considered to be a practical means of controlling the disease.

11. It is considered that control of the disease can best be effected by attention to field sanitation, land selection and fertilisation of the tobacco.

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